process of elongation, and also to their structural genes. These results have demonstrated the non-ribosomal peptide synthesis character of the type B components.--

Please replace the paragraph beginning on page 12, line 18, with the following paragraph:

--This or these genetic modification(s) alter(s) the expression of the said gene, that is render(s) this gene, and, as the case may be, another of the genes involved in the biosynthesis of the precursors, partially or totally incapable of encoding the natural enzyme which is involved in the biosynthesis of at least one precursor. The inability of the said genes to encode the natural proteins may be manifested either by the production of a protein which is inactive due to structural or conformational modifications, or by the absence of production, or by the production of a protein having an altered enzymatic activity, or else by the production of the natural protein at an attenuated level or in accordance with a desired mode of regulation. The totality of these possible manifestations is expressed by an alteration of, or perhaps a blockage in, the synthesis of at least one of the group B streptogramin precursors.--

Please replace the paragraph on page 13, lines 15-17, with the following paragraph:

--These genes are more preferably the papA (SEQ ID NO: 14), papM (SEQ ID NO: 16), papB (SEQ ID NO: 4), papC (SEQ ID No. 2), hpaA (SEQ ID NO: 12), snbF (SEQ ID NO: 9), and pipA (SEQ ID NO: 7) genes described below.--

Please replace the paragraph on page 14, lines 7-16, with the following paragraph:

--The sequence homologies demonstrated for the PapB (SEQ ID NO: 5) and PapC (SEQ ID NO: 3) proteins show that these proteins are also involved, jointly with the PapA (SEQ ID NO: 15) and PapM (SEQ ID NO: 17) proteins, in the biosynthesis of the DMPAPA precursor. The two corresponding novel genes, papB and papC, were isolated and identified by subcloning which was carried out using cosmid pIBV2, described in Patent Application PCT/FR93/0923, and a plasmid, pVRC900, which is derived from pIBV2 by means of a HindIII deletion and is also described in Patent Application PCT/FR93/0923.--

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FINNEGAN, HENDERSON,
FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N. W.
WASHINGTON, DC 20005
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Please replace the paragraph beginning on page 15, line 23, with the following paragraph:

--On comparing the protein encoded by the hpaA gene and the protein sequences contained in the Genpro library, a homology of from 30 to 40% was detected with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryCI, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by another route than that of cyclodeamination (see examples 1-2 and 2-1), probably requires a transamination step which can be catalyzed by the product of this gene termed hpaA (SEQ ID NO: 12). Furthermore, the results of mutating this gene demonstrate unequivocally that it is involved in the synthesis of the 3-HPA precursor.--

NE

Please replace the paragraph beginning on page 16, line 10, with the following paragraph:

--Comparison of the product encoded by the gene termed pipA with the protein sequences contained in the Genpro library shows a 30% homology with the ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step of the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of incorporating labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989, Reed et al., 1989). Cyclodeamination of lysine, in a similar manner to that described for ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, this product was termed PipA (SEQ ID NO: 7). The results of mutating the pipA gene, presented in the examples below, demonstrate that it is involved solely in the synthesis of pipecolic acid. It is noted, in particular, that this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.--

NE

Please replace the paragraph beginning on page 17, line 5, with the following paragraph:

--Finally, on comparing the product of the gene termed snbF with the protein sequences contained in the Genpro library, a 30 to 40% homology was noted with several hydroxylases of the cytochrome P450 type, which are involved in the biosynthesis of secondary metabolites (Omer et al., 1990; Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of the precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The corresponding protein was termed SnbF (SEQ ID NO: 9).--

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Please replace the paragraph beginning on page 17, line 28, with the following paragraph:

--Genetic modification should be understood to mean, more particularly, any suppression, substitution, deletion, or addition of one or more bases in the gene(s) under consideration. Such modifications may be obtained in vitro (on the isolated DNA) or in situ, for example, by means of genetic engineering techniques, or else by exposing the said microorganisms to a treatment using mutagenic agents. Examples of mutagenic agents which may be cited are physical agents such as high energy rays (X, γ, ultraviolet, etc. rays), or chemical agents which are able to react with different functional groups of the DNA bases, and, for example, akylating agents [ethyl methanesulphonate (EMS), N-methyl-N'-nitro-N-nitrosoguanidine, and N-nitroquinoline-1-oxide (NQO)], bialkylating agents, intercalating agents, etc. Deletion is understood to mean any suppression of a part or all of the gene under consideration. This deletion can, in particular, be of a part of the region encoding the said proteins, and/or of all or part of the promoter region for transcription or translation, or else of the transcript.--

Please replace the paragraph on page 21, beginning on line 8, with the following paragraph:

--Naturally, the novel precursor is such that it complements the alteration or blockage, which is induced in accordance with the invention, within the biosynthesis of one of the natural precursors of the group B streptogramins and leads to the synthesis of streptogramins. According to one particular embodiment of the invention, this novel precursor is selected such that it is related to the precursor whose biosynthesis is altered. Thus, in the specific case of the mutant which is blocked in the biosynthesis of DMPAPA, the novel precursor is preferably a derivative of phenylalanine.--

Please replace the paragraph on page 23, lines 16-20, with the following paragraph:

--The claimed process turns out to be particularly adventageous for preparing novel group B streptogramins or else for favoring formation of particular streptogramins. As such, it is particularly useful for preparing PI_B.--

Please replace the paragraph beginning on page 23, line 21, with the following paragraph:

--The present invention also relates to a nucleotide sequence which is selected from among:

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(a) all or part of the genes papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) and hpaA (SEQ ID NO: 12),

(b) sequences which hybridize with all or part of the (a) genes, and

(c) sequences which are derived from (a) and (b) sequences on account of the degeneracy of the genetic code.--

Please replace the paragraph on page 24, lines 3-6, with the following paragraph:

--In the particular case of the hybridizing sequences according to (b), these sequences preferably encode a polypeptide which is involved in the biosynthesis of the streptogramins.--

Please replace the paragraph on page 24, line 7, with the following paragraph:

--Still more preferably, the invention relates to the nucleotide sequences which are represented by the genes papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9), and hpaA (SEQ ID NO: 12).--

Please replace the paragraph on page 24, lines 12-15, with the following paragraph:

--The invention furthermore relates to any recombinant DNA which encompasses a papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) or hpaA (SEQ ID NO: 12) gene.--

Please replace the paragraph beginning on page 24, line 26, with the following paragraph:

--The present invention also relates to any mutated S. pristinaespiralis strain which possesses at least one genetic modification within one of the papC (SEQ ID No. 2), papB (SEQ ID NO: 4), pipA (SEQ ID NO: 7), snbF (SEQ ID NO: 9) and hpaA (SEQ ID NO: 12) genes, and, more preferably, to strains SP92pipA::Ωam^R and SP92hpaA::Ωam^R, as well as any *S. pristinaespiralis* strain, such as SP212, which possesses a genetic modification which consists of a disruption of the papA gene by means of double homologous recombination.--

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Please replace the paragraph on page 25, lines 8-22, with the following paragraph:

--Combinations of a component of the group A streptogramins and of a compound of the general formula I, according to the invention, constitute compositions which are particularly advantageous in the therapeutic sphere. They are employed, in particular, for treating ailments which are due to Gram-positive bacteria (of the genera Staphylococci, Streptococci, Pneumococci and Enterococci) and Gram-negative bacteria (of the genera Haemophilus, Gonococci, Meningococci). Thus, the compounds according to the invention have a synergistic effect on the antibacterial action of pristinamycin IIB on Staphylococcus aureus IP8203 in mice in vivo, at oral doses which are principally between 30 mg/kg and 100 mg/kg, when they are combined in PI/PII proportions of the order of 30/70.--

Please replace the paragraph on beginning on page 28, line 14, with the following paragraph:

--The clonings were carried out as follows. Approximately 2 µg of plasmid pVRC900 were cut with restriction enzymes PstI and/or XhoI (New England Biolabs) under the conditions recommended by the supplier. The restriction fragments thus obtained were separated on a 0.8% agarose gel, and the 1.5 kb PstI-PstI, 0.7 kb PstI-XhoI and 0.7 kb XhoI- XhoI fragments of interest were isolated and purified using Geneclean (Bio101, La Jolla, California). For each cloning, approximately 10 ng of M13mp19 and/or M13mp18, cut with PstI and/or XhoI, were ligated to 100 ng of the fragment to be cloned under the conditions described by Maniatis et al. 1989. After transforming the strain TG1 (K12, Δ(*lac-pro*) *supE thi hsd* ΔS F' *tra*D36 *pro*A^+B^+ *lac*I^q *lac*Z Δ M15; Gibson, 1984) and selecting lysis plaques on an LB + X-gal + IPTG medium in accordance with the technique described by Maniatis et al. (1989), the phage carrying the desired fragments were isolated. The different inserts were sequenced by the chain termination reaction using, as the synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. The reactions were carried out using fluorescent dideoxynucleotides (PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit-Applied Biosystem) and analysed on a model 373 A Applied Biosystems DNA sequencer. The overlap between these different inserts was such that it was possible to establish the entire nucleotide sequence between the papA and papM genes (SEQ ID No. 1).--

Please replace the paragraph on page 31, lines 9-22, with the following paragraph:

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1300 I STREET, N. W.
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--Comparison of the product of frame 2 (TABLE I) with the protein sequences contained in the Genpro library shows a 27% homology with the region involved in the prephenate dehydrogenase activity of the bifunctional TyrA proteins of E. coli (Hudson and Davidson, 1984) and of Erwinia herbicola (EMBL data library, 1991). This region of TyrA catalyses aromatization of prephenate to form 4-hydroxyphenylpyruvate in the biosynthesis of tyrosine. A similar aromatization, proceeding from 4-deoxy-4-aminoprephenate and leading to 4-aminophenylpyruvate is very probably involved in the synthesis of DMPAPA. This reaction will be catalyzed by the product of frame 2, termed PapC (SEQ ID NO: 3).--

Please replace the paragraph beginning on page 31, line 23, with the following paragraph:

--Comparison of the product of frame 3 (TABLE I) with the protein sequences contained in the Genpro library shows a 24 to 30% homology with the region involved in the chorismate mutase activity of the bifunctional TyrA and PheA proteins of E. coli (Hudson and Davidson, 1984) and of the TyrA protein of Erwinia herbicola. This region catalyzes isomerization of chorismate to form prephenate in the biosynthesis of tyrosine and phenylalanine. A similar isomerization, proceeding from 4-deoxy-4-amino chorismate and leading to 4-deoxy-4-aminoprephenate, is very probably involved in the synthesis of DMPAPA. This reaction would be catalyzed by the product of frame 3, termed PapB (SEQ ID NO: 5).--

Please replace the paragraph on page 33, lines 20-23, with the following paragraph:

--The overlap between these different inserts enabled the entire nucleotide sequence which is present between the snbA and papA genes (SEQ ID NO: 6) to be established.--

Please replace the paragraph beginning on page 35, line 9, with the following paragraph:

--Comparison of the product of frame 2 (TABLE II) with the protein sequences contained in the Genpro library shows a 30% homology with ornithine cyclodeaminase of Agrobacterium tumefaciens (Schindler et al., 1989). This enzyme is involved in the final step in the catabolism of octopine; it converts L-ornithine into L-proline by means of cyclodeamination. Authors have demonstrated, by means of the incorporation of labelled lysine, that 4-oxopipecolic acid and 3-hydroxypicolinic acid, which are found both in PI_A and in virginiamycin S1, derived from lysine (Molinero et al., 1989; Reed et al., 1989). A reaction in which lysine was cyclodeaminated, similar to that described for

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ornithine, would lead to the formation of pipecolic acid. Taking this hypothesis into account, the product of frame 2 was termed PipA (SEQ ID NO: 8). The results of mutating the pipA gene, presented in 2-1, demonstrate that the pipA gene is involved solely in the synthesis of pipecolic acid, since this mutation has no effect on the biosynthesis of 3-hydroxypicolinic acid, which is also derived from lysine and of which pipecolic acid could have been a precursor.--

Please replace the paragraph on page 36, lines 8-24, with the following paragraph:

--Comparison of the product of frame 3 (TABLE II) with the protein sequences contained in the Genpro library shows a 30 to 40% homology with several hydroxylases of the cytochrome P450 type, which hydroxylases are involved in the biosynthesis of secondary metabolites (Omer et al., 1990, Trower et al., 1992). Several hydroxylations can be envisaged in the biosynthesis of precursors of pristinamycin I, in particular in the biosynthesis of 3-HPA (hydroxylation of picolinic acid at the 3 position) and of 4-oxopipecolic acid (hydroxylation of pipecolic acid at the 4 position). The results of mutating the pipA gene, presented in 2-1-3, demonstrate that the product of frame 3 is involved in hydroxylation of the pipecolic acid residue of PI_E. The corresponding gene has therefore been termed snbF, and the corresponding protein SnbF (SEQ ID NO: 9 and SEQ ID NO: 10, respectively).--

Please replace the paragraph beginning on page 37, line 8, with the following paragraph:

--The 1.6 kb HindIII-BglII fragment was subcloned into the M13mp18 and M13mp19 vectors, proceeding from cosmid pIBV2. The insert was sequenced as described in 1-1, using, as synthesis primer, the universal primer or synthetic oligonucleotides which were complementary to a 20 nucleotide sequence of the insert to be sequenced. On the basis of the nucleotide sequence thus obtained (SEQ ID NO: 11), it is possible to determine the open reading frames and to identify, in S. pristinaespiralis, genes which are involved in the biosynthesis of the precursors of PI, as well as the polypeptides encoded by these genes. We looked for the presence of open reading frames within the 1.6 kb HindIII-BglII fragment, which corresponds to the end of the snbA gene and its downstream region, as described in Example 1-1. A complete open coding frame, encoded by the same strand as the snbA gene (Figure 6), was detected. Relative to position 1, corresponding to the HindIII site, this frame starts at nucleotide 249, i.e. 30 nucleotides after the end of the snbA gene, and terminates at nucleotide 1481. It is 1233 nucleotides in size, corresponding to a protein of 411 amino acids.--

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Please replace the paragraph on page 38, lines 2-16, with the following paragraph:

--Comparison of the product of this open frame with the protein sequences contained in the Genpro library shows a 30 to 40% homology with a group of proteins which are probably involved (Thorson et al., 1993) in the transamination of intermediates in the biosynthesis of various antibiotics (DnrJ, EryC1, TylB, StrS and PrgL). Synthesis of the 3-HPA precursor, which appears to derive from lysine by a route other than cyclodeamination (see Examples 1-2 and 2-1), could necessitate a transamination step which can be catalyzed by the product of this frame 3, termed HpaA (SEQ ID NO: 13). The results of mutating this gene, presented in 2-2, demonstrate unequivocally that this gene is involved in synthesis of the 3-HPA precursor and confirm our hypothesis.--

Please replace the paragraph beginning on page 46, line 5, with the following paragraph:

--Mutant SP92pipA::Ωam^R, as well as strain SP92 in the role of a control strain, were cultrured in liquid production medium. The fermentation was carried out as follows: 0.5 ml of a suspension of spores from the abovementioned strain are added, under sterile conditions, to 40 ml of inoculum medium in a 300 ml baffled Erlenmeyer flask. The inoculum medium is made up of g/l corn steep, 15 g/l sucrose, 10 g/1 $(NH_4)_2SO_4$, 1 g/l K_2HPO_4, 3 g/l NaCl, 0.2 g/l $MgSO_4$-$7H_2O$ and 1.25 g/l $CaCO_3$. The pH is adjusted to 6.9 using sodium hydroxide solution before introducing the calcium carbonate. The Erlenmeyer flasks are shaken at 27°C for 44 h on a rotating shaker at a speed of 325 rpm. 2.5 ml of the previous culture, which is 44 hr old, are added under sterile conditions to 30 ml of production medium in a 300 ml Erlenmeyer flask.. The production medium is made up of 25 g/l soya flour, 7.5 g/l starch, 22.5 g/l glucose, 3.5 g/l fodder yeast, 0.5 g/l zinc sulphate and 6 g/l calcium carbonate. The pH is adjusted to 6.0 with hydrochloric acid before introducing the calcium carbonate. The Erlenmeyer flasks are shaken for 24, 28 and 32 hours at 27°C. At each time point, 10 g of must are weighed into a smooth Erlenmeyer flask to which 20 ml of mobile phase, consisting of 34% of acetonitrile and 66% of a solution of 0.1 M KH_2PO_4 (adjusted to pH 2.9 with concentrated H_3PO_4) are added for extracting the pristinamycins. After shaking, the whole is centrifuged and the pristinamycins contained in the supernatant are assayed by HPLC by means of injecting 150 µl of the centrifugation supernatant onto a Nucleosil 5-C8 column of 4.6 x 150 mm, which is eluted with a mixture of 40% acetonitrile and 60% 0.1 M phosphate buffer, pH 2.9. The I pristinamycins are detected by means of their UV absorbance at 206 nm.--

Please replace the paragraph beginning on page 59, line 19, with the following paragraph:

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--The following table (TABLE IV) indicates the relative retention times of the new PI's which are produced, taking PI_A as the reference. The absolute retention times were determined at 25°C in the HPLC system described above; they vary slightly from one injection to another and also in accordance with temperature.--

On page 121, line 14, please delete "[lacuna]" and insert therefor:

--8 Hz, 1H: aromatic H in the meta position with respect to the dimethylamino); 7.43 (limiting AB, 2H: 1'H_4 and 1'H_5); 7.82 (mt, 1H: 1'H_6); 8.38 (d, J = 10 Hz, 1H: NH in 1); 8.73 (d, J = 9.5 Hz, 1H: NH in 6); 11.61 (s, 1H: OH).

EXAMPLE 29: Preparation of 4ϵ-methylthio-de(4ζ-dimethylamino)pristinamycin I_A

Strain SP92::pVRC508 is cultured in production medium using 56 erlenmeyer flasks, as described in Example 3, with 1--.

Please replace the paragraph on page 133, line 20, with the following paragraph:

--J.I. Degaw et al., J. Med.Chem., 1969, 11, 225-227--

Please replace the paragraph on page 133, line 26, with the following paragraph:

--Y. Sasaki et al., Chem. Pharm. Bull., 1982, 30, 4435--

Please replace the paragraph on page 134, line 2, with the following paragraph:

--A. Zhuze et al., Coll., Czech. Chem. Comm., 1965, 62, 2648--

Please replace the Sequence Listing on pages 171-188 with the enclosed paper copy of the Substitute Sequence Listing filed in parent U.S. Application No. 08/765,907 on July 25, 2001.

IN THE CLAIMS:

Please delete claims 1-29 without prejudice or disclaimer thereof, and add new claims 30-57 as follows:

30. (New) A compound of formula I

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FARABOW, GARRETT,
& DUNNER, L.L.P.
1300 I STREET, N.W.
WASHINGTON, DC 20005
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